

Getting Started



Thank you for purchasing SlideBook 6!

This Getting Started Guide was designed to familiarize you with the layout and basic functionality of SlideBook 6 software. The content in this guide can also be found in the software help system, by pressing F1 or  at any time.

Kindest Regards,
3i Support Team

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If you have any questions or experience any problems with SlideBook™, please contact our support team:

By Phone: 303.607.9429 ext.2

By Email: support@intelligent-imaging.com

On the web: <http://www.intelligent-imaging.com>



Getting Started with SlideBook

A series of tutorials have been provided to aid in getting familiar with the tools provided in SlideBook. These tutorials walk you through basic operations provided in the software. Alternatively, each user interface window has a help page associated with it that diagrams each function and advises in how to use the options available.

For tutorials and user interface pages, the following typographic conventions are used:

Menu commands, dialog fields, and other permanent interface items are written in bold italics and follow the order of menu navigation. For instance, choosing "Open" from the "File" menu is written as ***File > Open***.

NOTES are written in bold and capitals.

A stylized font is used for user-defined fields or hot key shortcuts such as **file names** or **text entries** or press **Delete**.

References to other sections of the help are highlighted such as [Focus Controls Window](#).

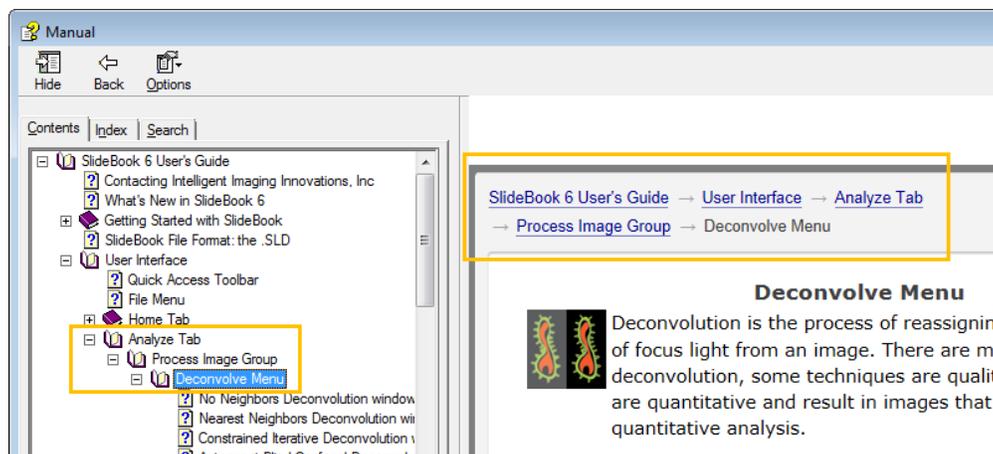
SlideBook is organized using a graphical menu and icon system. There is a customizable **Quick Access toolbar** across the top of the application with the rest of the program functionality being found in a tabbed system. The tabs are designed to group functionalities and improve workflow while minimizing the number of clicks required to accomplish tasks.



Terminology used in the manual refers to location by tab and group and often includes an image of the icon. A main portion of this help system is the User Interface section. User interface pages are organized in a structure that mimics the actual user interface:

Tab > Group > Menu/Icon > Function

When you are on a specific user interface page of the help system, the location of the page can be found in the organizational tree on the left side of the Help pane, or across the top of the window.



Tabs are the largest organizational feature of SlideBook. Tabs available include:

File - execute normal file functions such as open, close, create and save **.sld**

Home - control hardware to set up and acquire images, import and export images, view images using basic and advanced techniques, and access region tools and annotations

Analyze - deconvolve and filter data, perform mask generation and analysis, graph, particle track, smooth curve analysis and analyze using other techniques

Scripting - execute, design, and edit existing scripts

Modules - access purchased analysis modules such as FRET, Ratio, FLIM, and Stereology

Support - easily update software and key, backup configuration, contact 3i technical support

Administration - resource for system administrators that includes setup, maintenance, and configuration tools

In the **Home** tab, you will find two main hardware control icons to open **Focus** control and **Capture** control dialogs. These two dialogs are all you need to set up anything from a simple 2D single channel image to a 3D multi-channel capture that visits multiple x,y locations over time (6D capture).



Focus controls allow you to arrange the sample and examine the specimen interactively.



Capture controls allow you to set up parameters for automated image capture.



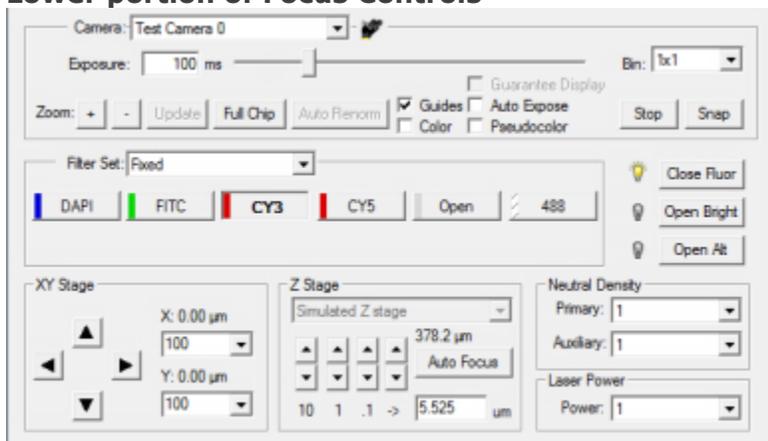
Introduction to Focus Controls

Focus controls are accessed from the  **Focus** icon in the Home tab, or by pressing **Ctrl + F**.

The Focus Controls window is used to help arrange and view the specimen before setting up an acquisition. You can use the Focus Controls to center the specimen relative to the camera, check fluorescence levels, take a 2D single channel snapshot, adjust camera parameters, or set up a point list for multi-point visiting experiments.

The lower portion of the window is static while the top portion of the window has different tabs for specific functionality. The upper portion of the window changes with regards to control modules available. We will first examine the lower portion of the window as it is used intermittently with tabs of the top portion.

Lower portion of Focus Controls



Adjust exposure time, binning, and appearance of live image window.

"Snap" a 2D single channel image.

Select filter sets for viewing.

Open and close shutters.

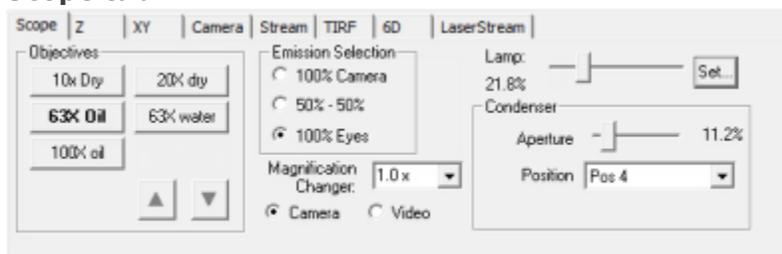
Move the stage in X, Y, or Z.

Adjust movement size.

Test the "Auto Focus".

Neutral density settings.

Scope tab

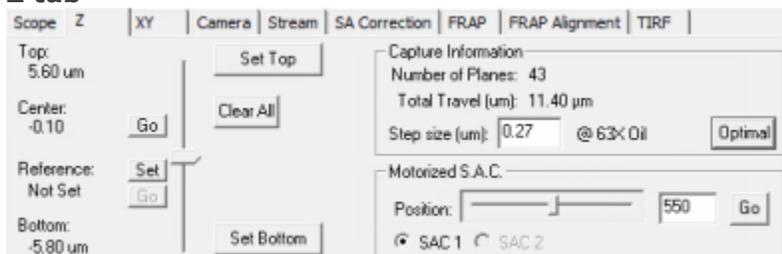


Select objective.

Direct light to eyes or camera.

Adjust transmitted light intensity and condenser.

Z tab

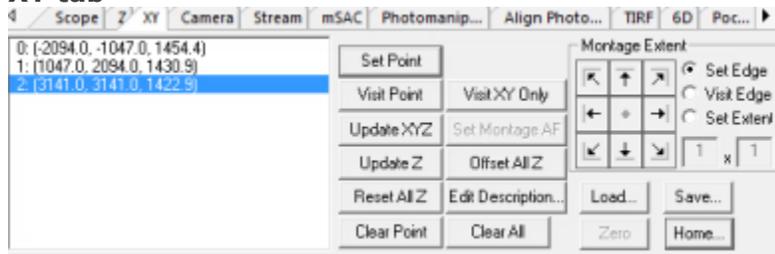


Set top and bottom of z range for acquisition.

"Go" to center of range.

Set z step size for capture.

XY tab

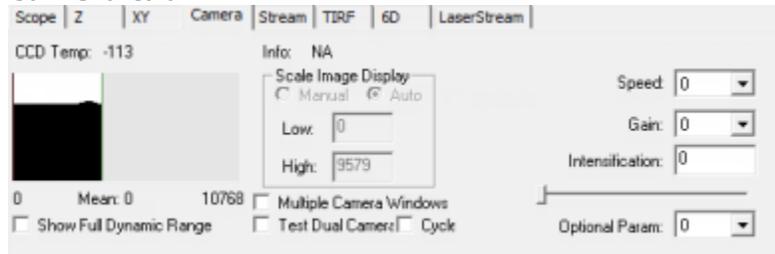


Set x,y,z positions for point visiting experiments.

Set extents for montage imaging.

Edit points.

Camera tab



View intensity information based on sensitivity and exposure time.

Choose manual or auto-scaling display for live window.

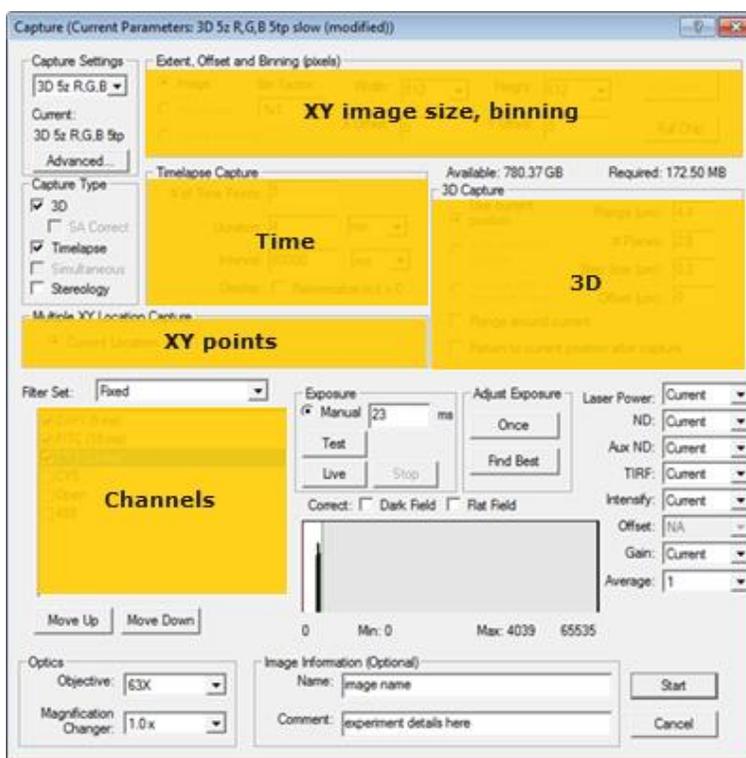
Adjust intensification, speed, and gain for camera readout.



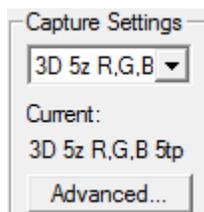
Introduction to Capture Controls

Capture controls are accessed from the  **Capture** icon in the **Home** tab, or by pressing **Ctrl + E**.

The Capture Controls dialog provides all necessary tools to set up basic or complex capture sequences. The dialog is broken down into areas to adjust parameters for different dimensions of capture as depicted below.



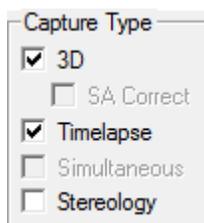
Capture Settings



Save/Load Capture Settings

Access **Advanced** options including variable time intervals, photomanipulation, autofocus, periodic imaging, speed optimizations, and triggering.

Capture Type



Select elements to be included in image acquisition then complete relevant fields.

If none of these elements is desired, proceed to Channel Selection and Exposure Time.

Extent - Offset - Binning

Extent, Offset and Binning (pixels)

Image Bin Factor: Width: Height:

Autofocus X Offset: Y Offset:

White Balance

Select a bin factor to increase signal (note this will decrease resolution). Manually select a chip size based on the center of the chip.

OR

Draw a region to image on the live window using the tool. Click "Update" to apply.

Timelapse Capture

Timelapse Capture

of Time Points:

Duration:

Interval:

Display: Renormalize to t = 0

Enter parameters for time series acquisition.

Multiple XY Location Capture

Multiple XY Location Capture

Current Location Multipoint List

Select a multipoint list if you would like to perform point visiting or capture images for a montage.

3D Capture

Available: 780.45 GB Required: 122.20 MB

3D Capture

Use current position Range (um):

Use reference position # Planes:

Use top and bottom positions Step Size (um):

Range around current Offset (um):

Return to current position after capture

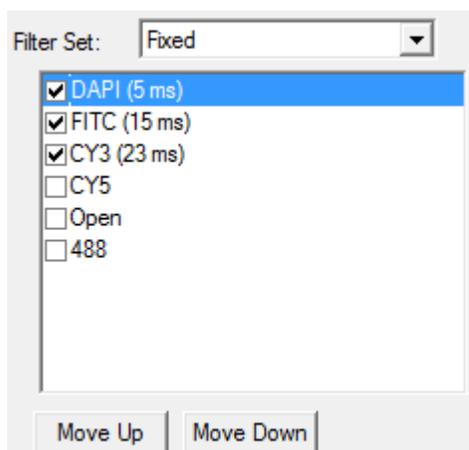
Select elements to be included in image acquisition then complete relevant fields.

If none of these elements is desired, proceed to Channel Selection and Exposure Time.

Be aware of the amount of memory available and required for the capture. It may be necessary to adjust the portion of the chip used, binning, number of time points, or number of Z sections to create a manageable data set.



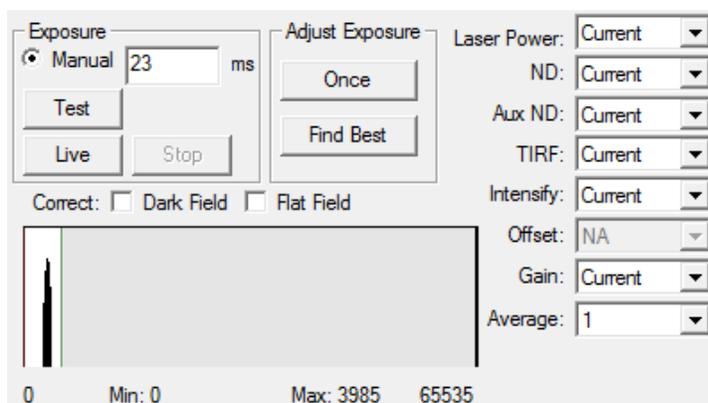
Channels



Check the boxes next to the imaging filters you would like to capture.

Use the buttons below to adjust the order in which the channels are captured.

Exposure



Adjust the exposure time for each channel that will be captured. Use the **Live** button should you need to reposition your sample.

Refer to the histogram (see tips below) to determine optimal exposure times.

Try using the buttons in the adjust exposure dialog to allow SlideBook to optimize exposure time for your sample.

Adjust neutral density settings, gain, and intensification for the camera as desired. Use "Current" to use the settings from the focus controls. Different settings can be applied to each channel.

Setting Exposure Time

Setting exposure time appropriately is essential to obtaining the desired imaging results, for live cell experiments, it can be a matter of life and death! When using an EM (electron multiplying) camera, one needs to adjust intensification with exposure time. Here are some basic tips for setting exposure time and intensification appropriately.

IDEAL



ACCEPTABLE FOR LIVE CELL



SATURATED



UNDEREXPOSED



TIPS

For fixed specimens, aim for filling the histogram 2/3 of the way. This gives you room for brighter planes.

Never saturate an image you would like to use for deconvolution or most other quantitative analysis techniques.

For live samples, experiment to determine how much light they can handle. You will often sacrifice intensity for cell viability. It is acceptable to have your signal at twice the background level, depending on the noise in the image.

When imaging live samples with an EM camera, you can increase the signal while keeping the exposure time low by increasing the intensification.

If possible, try to keep the intensification level well below the maximum available.

When using a non-EM, higher resolution camera, another way to increase signal without increasing exposure time is to utilize binning. It is important to note that binning will decrease the lateral resolution of an image.



Example Experiment

This section will step through the process of setting up an example data capture. For brevity, we will walk you through a 4D multipoint capture setup. For less complex experiments, simply exclude the aspects of the experiment that are not relevant to your work.

Before You Begin

If you are using an incubation chamber, it should be turned on at least one hour before you begin setting up your experiment. Immersion media for objectives such as distilled water or the microscope manufacturer's immersion oil should be placed in the chamber and allowed to come to temperature as well. It is important that the temperature of the equipment has stabilized to eliminate drift artifacts in the data capture.

Ensure the objective lens is clean and in the lowered (raised for upright microscopes) position. If the objective requires cleaning, use lens tissue or 100% cotton swabs and an approved solvent. Never use general purpose lab or household tissue on optical surfaces. Once the objective is clean, apply the appropriate immersion media*. Ensure there are no bubbles in the media. If there are bubbles during application, remove the fluid using lens tissue and re-apply.

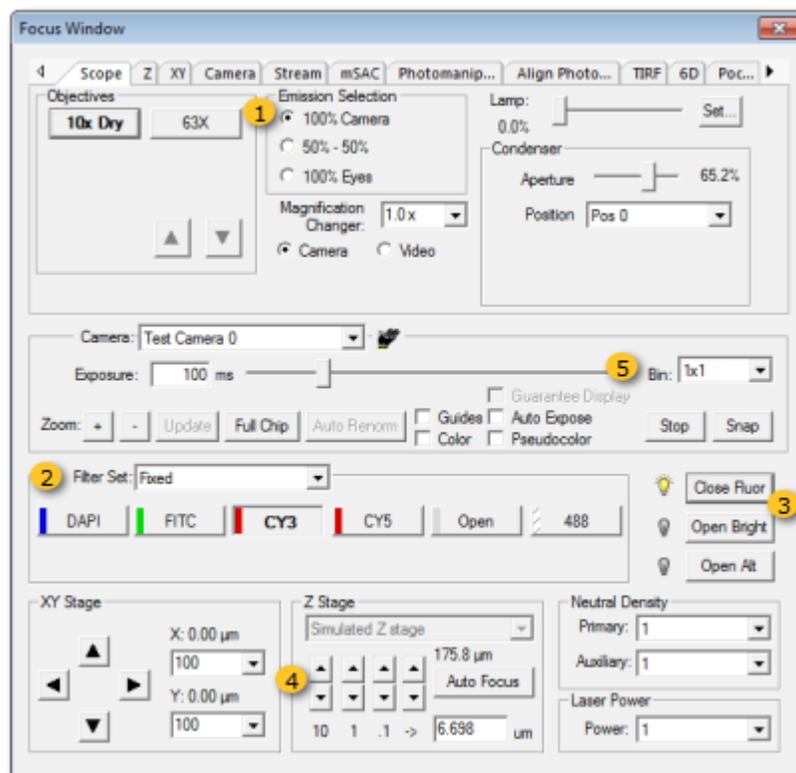
Arrange the specimen on the microscope stage with the coverslip toward the objective lens. Bring the objective toward the sample. For air objectives, stop at about 1mm from the coverslip. For water and oil objectives, stop once the media has contact with the coverslip. Use stage clips, the stage insert, or lab tape to secure the specimen to the stage. Direct light to the specimen and bring the sample into focus looking through the oculars.

* It is important to use the microscope manufacturer's immersion oil to maintain the chromatic properties of high end objectives. For upright microscopes, the immersion media will be applied to the coverslip of the specimen rather than the objective. Alternately, many upright microscopes have "dipping" lenses that can be inserted directly into the specimen's media.

Open Focus Controls, Orient Specimen



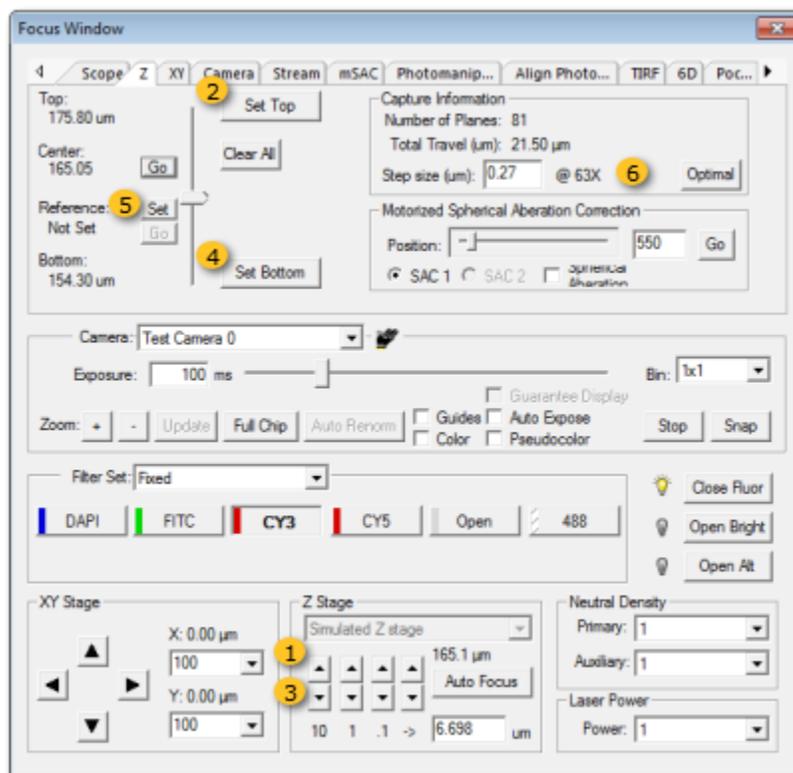
1. In the **Scope** tab of **Focus** Controls, set **Emission Selection** to **100% Camera**.
2. Select the filter set and channel you would like to preview.
3. Open the appropriate shutter to direct light to the sample.
4. Adjust the focus using the knobs on the microscope or z stage controls to bring the specimen into focus.
5. If necessary, use binning or change exposure time to alter the appearance of the image on the screen. You will set parameters for final capture later.





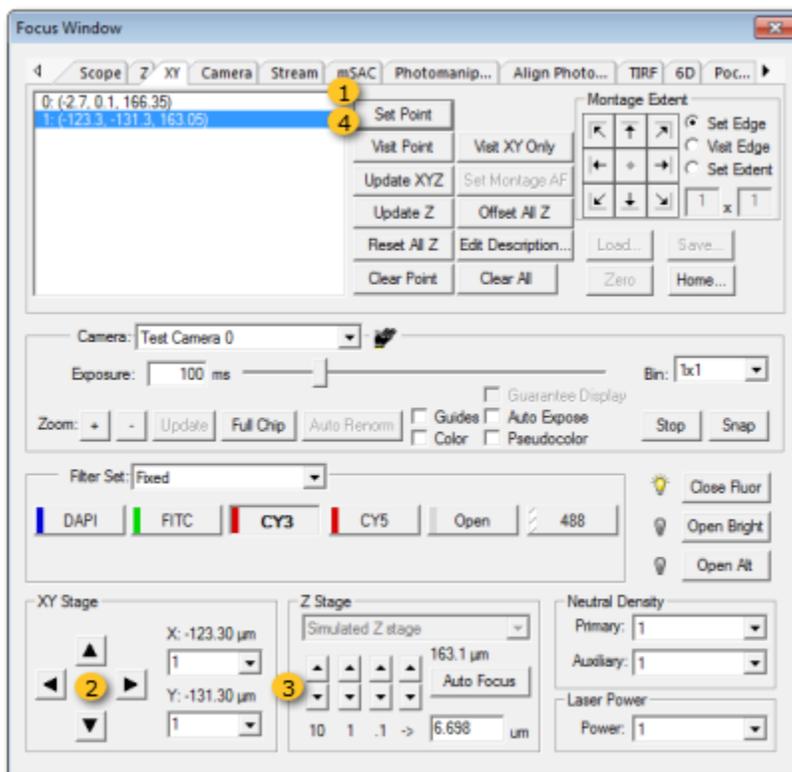
Set Z Extents

1. Navigate to the top z extent using the z stage controls.
2. Click **Set Top**.
3. Navigate to the bottom extent using the z stage controls.
4. Click **Set Bottom**.
5. Click **Go** to return to the center of the stack.
6. Set the step size for data collection or click **Optimal** to use Nyquist sampling of the theoretical axial resolution of the objective.



Set Points for Visiting

1. Click **Set Point** to add the current position to the list for capture.
2. Navigate to the next point using the joystick for the motorized stage or the stage controls.
3. Adjust the z focus to the center of the stack using the focus knob or the z stage controls.
4. Click **Set Point** to add the next point to the list.
5. Repeat steps 2–4 until all desired points are set.





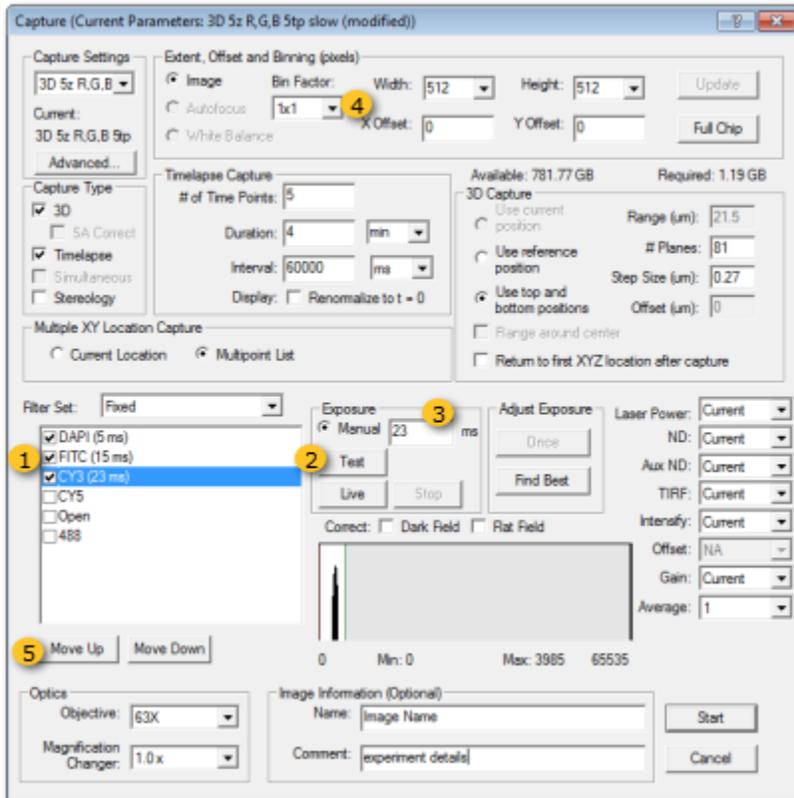
Open Capture Window, Set Exposure Times



1. Open the **Capture** dialog, check the box next to the channel you would like to capture.
2. Click **Test**.
3. Adjust **Exposure** parameters [see notes [here](#) for additional information on setting exposure parameters.]
4. If desired, adjust bin factor to further increase signal, adjust exposure time and intensification accordingly. Repeat 1–3 for additional imaging channels, noting that only one bin factor can be used per experiment.

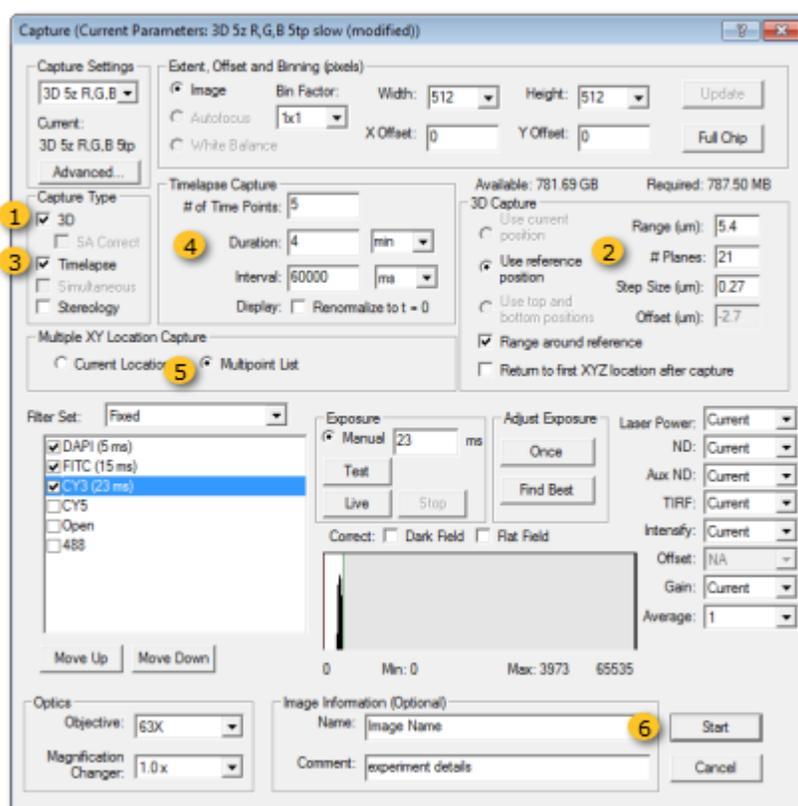
NOTE: Binning reduces xy resolution.

6. Select channels and use the **Move** buttons to change capture order.



Set Capture Parameters, Start Acquisition

1. Check the box for **3D**.
2. Select the type of 3D capture to perform. For multipoint experiments, select **Use reference position** and **Range around reference**. For standard 3D imaging, you will likely select **Use top and bottom positions**.
3. Check box for **Timelapse**.
4. Enter **Timelapse Capture** details as desired.
5. Select the radio button for **Multipoint List** to direct the capture to the points marked in the XY tab of the Focus Controls.
6. Enter a **Name** for the image if desired. Click **Start**.





SlideBook File Format: the .SLD

A *slide* is SlideBook's native file type (*.sld). A slide file corresponds to a single file on the disk and can be thought of as SlideBook's document type. It can contain many multi-plane, multi-channel, multi-timepoint images in any combination.

Double-clicking on an image in a slide will open a **Main View** of the image.

You may also right-click on an image and perform the following functions: rename, cut, copy, paste, delete. Additionally, you may select **Properties** to view information about the image capture. **Recapture Experiment** is available for images captured in SlideBook 6 and later. By selecting **Recapture Experiment**, the **Capture** dialog will open, populated with the experiment acquisition parameters used in collecting the selected image, thus easily reproducing the acquisition.

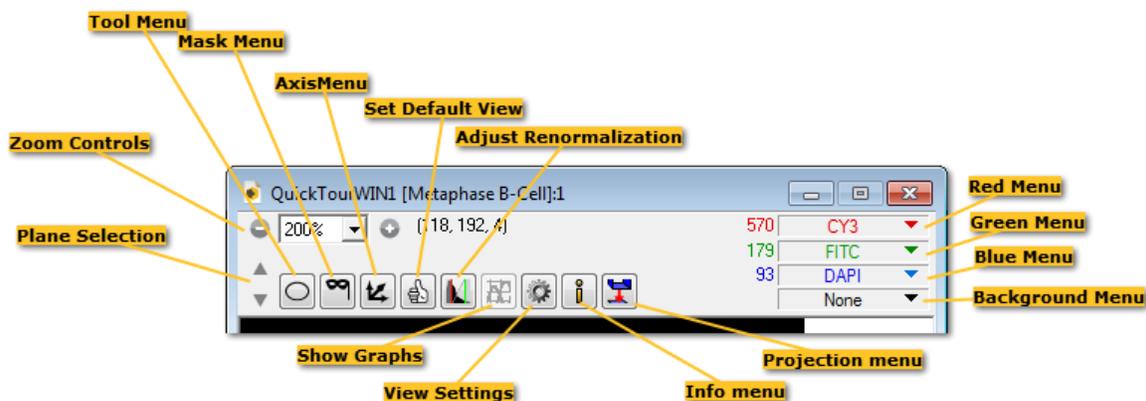
In the **.sld**, each image will be represented as a thumbnail with associated image information including comments made during capture or analysis, capture date, capture type, number of channels, physical image dimensions, amount of drive space taken up by image (size), and number of masks. The thumbnail style represents the capture type. Three dimensional images will be represented as a stack of thumbnails. Images taken over time will have a film strip depiction above the thumbnail. The image seen in the thumbnail is a direct representation of the default view of the image. To adjust how the thumbnail appears in a **.sld**, open the image and adjust the plane, renormalization, lookup table, etc. Once

the image appears as desired, click on the thumb icon  and **Save** the **.sld** to set the default setting for future openings of the slide and images.

A **.sld** must be open in order to run the **Focus Window** or set up image capture.

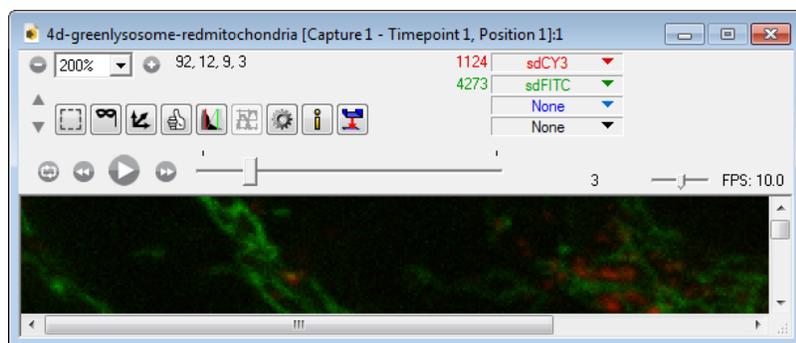
TUTORIAL: Viewing an Image

When you open an image by double-clicking on the thumbnail in a slide, a Main View will open. Alternatively, you may open a Main View or a Three View from the View Menu. All views contain many useful tools outlined below.



In any open view, the middle mouse scroll wheel will scroll through either z-planes or time. In a 4D series the scroll wheel will control time or you may hold down the **CTRL** key while scrolling the mouse wheel to change z-planes.

When an image consists of multiple time points, additional tools will appear in the Main View. We will briefly review the tools associated with playing back time lapse images.



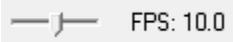
The *Playback Controls* allow you to toggle looping/single playthrough, move back one time point, play/pause, or move forward one time point.



The *Time Slider* displays the current time point both with the small gray box and with a numerical display to the right of the slider bar. In the example above, the user is at time point 3, about 20% of the way through the entire time series. A user can move to a desired time point by clicking on the small gray box and dragging it to the desired time point. When the time slider control is selected, it will be surrounded by a dotted line box. Once selected,



you can move forward and backward through time by using the middle scroll button on the mouse.



The *Playback Speed Slider* works in a manner similar to the time slider. Instead of adjusting the time point, this slider controls the speed of the image looping in *FPS* or frames per second.

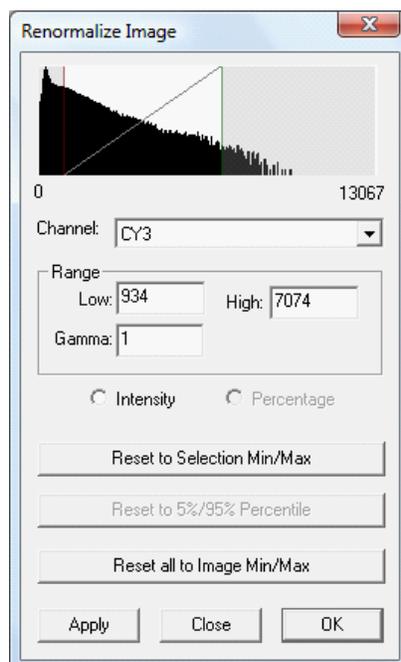
TUTORIAL: Adjusting Display of an Image

SlideBook offers some basic display tools for adjusting how data is represented on the computer screen.

Renormalization

Renormalization allows you to alter the range of intensities displayed for each channel. To open the Renormalize Image window, click on the **Adjust LUT** icon  in the **Home** tab or **View** window.

1. Open the **Renormalize Image** window:



A histogram is displayed showing the relative number of pixels on the y axis and intensity values on the x axis for the specified channel. In this image, the CY3 channel intensities range from 0 to 13067.

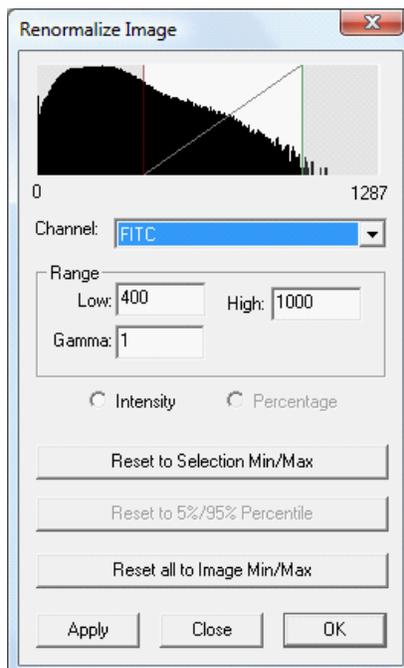
2. Click on the drop-down **Channel** menu and select the channel you would like to adjust.
3. Click on the red and green bars in the histogram window and drag them to the left or right, observing the change in the display of the relevant channel data.

The red and green bars allow you to select the minimum and maximum intensities that correspond to the absence (black) or full saturation of color in the display, respectively. Again, the underlying values in the image are not affected.

4. Now manually enter numbers in the **Low** and **High** data entry fields. Note that the image does not automatically update as it does when moving the red and green bars.
5. Click **Apply** to register the change and update the image.



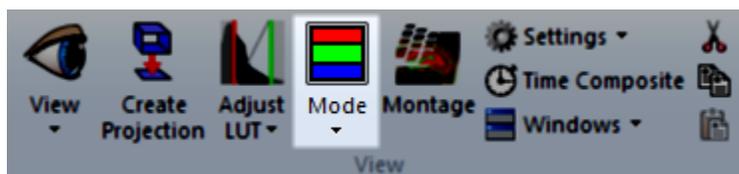
6. Press **Reset all to Image Min/Max** to set the minimum and maximum display values to the minimum and maximum pixel values.



7. Choose **OK** to exit the Renormalize Image dialog box.

Change the Color Scheme

You may also change the basic way channels are displayed in the view window. For example, data can be displayed using a temperature lookup table or in monochrome. Shortcut keys are available for each of the option below or you may find them under the **Mode** menu in the **Home** tab.



RGB Color - **Ctrl + Shift + R** - Provides three colors (red, green, and blue) for assignment to any of the channels, also enables a background channel to blend a transmitted light image



Monochrome - **Ctrl + Shift + M** - Provides a single channel to display a monochrome image



Inverted Monochrome - **Ctrl + Shift + V** - Provides a single channel to display an inverted monochrome image



Pseudocolor - **Ctrl + Shift + P** - Provides a single channel to display on a hot/cold lookup table, may be gated by a second channel as in ratio data



User-defined Color - **Ctrl + Shift + D** - Provides up to eight channels which can be assigned to a user-defined color selected from a color chart or manually created

When a transmitted light, greyscale image is collected along with fluorescence, you can combine the channels using **Blend Background** (available under  **Settings** in the **View** group of the **Home** tab. Blend background is compatible with RGB Color and Pseudocolor display types. Degree of blending can be adjusted in the **View Settings** window.

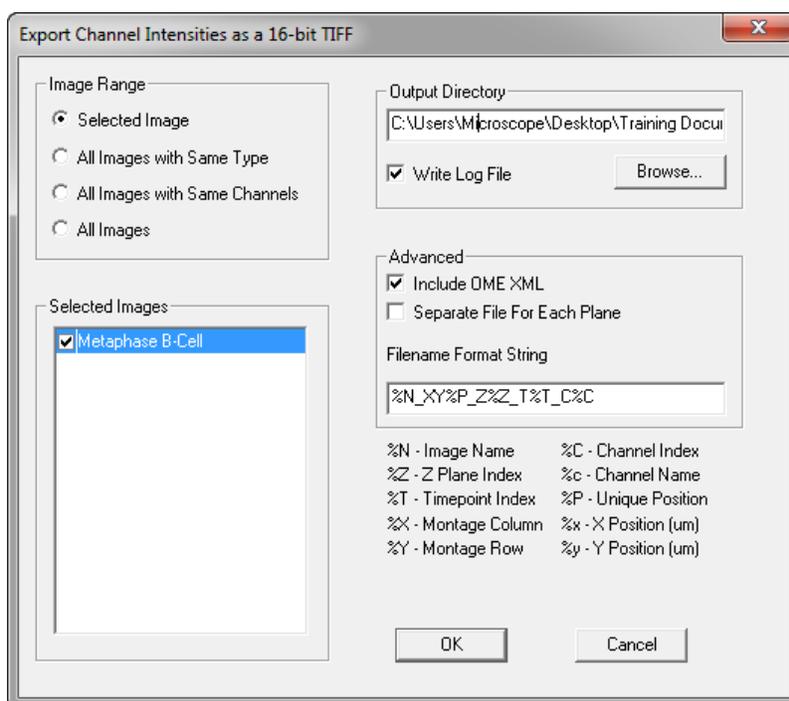


TUTORIAL: Quantitative Image Export

Sometimes, you will wish to share data with colleagues or back up data in a format that can be read by programs other than SlideBook. In order to do this, you will want to maintain the true intensity values of each pixel of the image. For this reason, there is a 16-bit tiff export option available (see also [16-bit Tiff Export window](#)). This export option is compatible with the OME (Open Microscopy Environment) format.

1. Select the image that you would like to export by clicking on it. You may also start from an open view of the image.

2. In the **Home** tab, select  **Export > (Quantitative Export) 16-bit TIFF**. A dialog similar to the following will appear.



3. Select the **Image Range** as described below. You may also select and deselect images using the checkboxes in the **Selected Images** list.

Selected Image – Operation will be performed on the selected image only.

All Images with Same Type – Operation will be performed on all images in the slide with the same capture type (2D, 3D, 4D, etc.) as the selected image.

All Images with Same Channels – Operation will be performed on all images in the slide with the same channels as the selected image.

All Images - Operation will be performed on all images in the slide.

4. Select the **Output Directory** where the tiffs will be saved by clicking on **Browse** then navigating to the desired location.

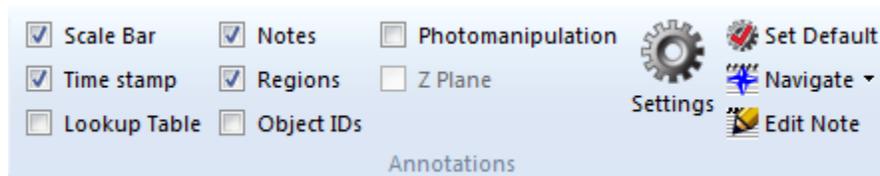
-
5. Check the box for ***Include OME XML*** to include OME metadata in the tiff file (encoded in XML).
 6. Check the box for ***Separate File For Each Plane*** if you would like a tiff file for each plane for each channel of the image. If you do not check this box, a multi-plane tiff file will be created for each channel.
 7. If desired, format the naming of the tiffs in the ***Filename Format String*** field.
 8. Press ***OK***.



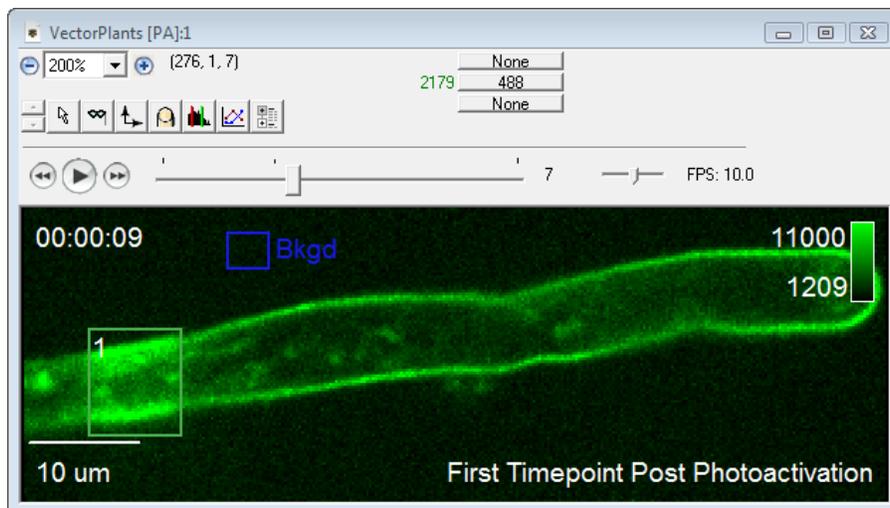
TUTORIAL: Displaying and Formatting Annotations

In any of the image views available, SlideBook provides tools to add annotations to displayed images. When annotations are shown in an image, they will be exported with any **Qualitative** export of the image. Annotations are not displayed when images are exported using a **Quantitative** technique. Additional information on these functions can be found in the [Annotations Group](#).

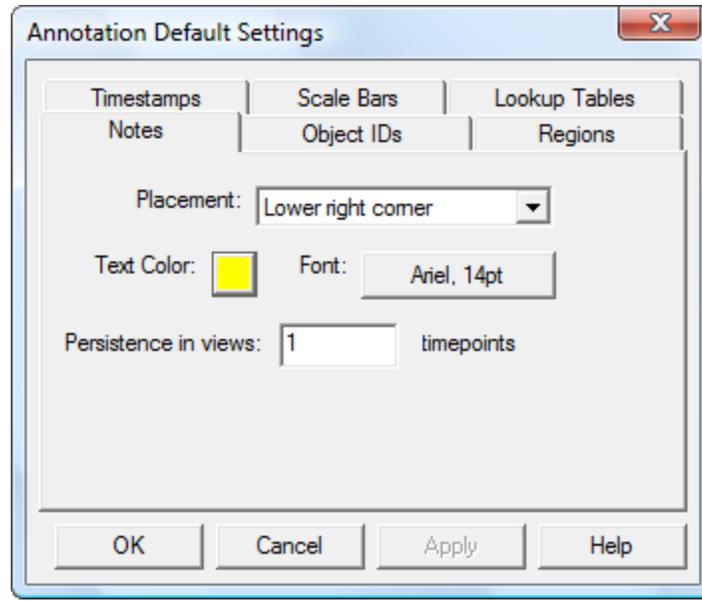
1. Open a view of the image you would like to annotate.
2. Go to the **Annotations** group in the **Home** tab.



3. Select the annotations you would like to display by checking the boxes. Alternatively, you can use shortcut keys.



4. Once the annotations are displayed, you may find it desirable to adjust the positioning or appearance of the annotations. To do this, select the **Settings**  icon in the **Annotations** group.



5. This dialog consists of a number of tabs that allow for formatting of each individual annotation. Users can adjust the color, placement, and font used for each annotation. Some annotations have annotation specific settings such as the length setting for scale bars. Adjust all annotations as desired then click **OK**. The changes will be made in your image window. It may be necessary to click once on the image for the updates to take place.

6. If you are only interested in adjusting annotations for the image you are working with, close the window when finished. Should you choose to apply these annotation settings to every image you open, it is possible to set the default behavior for annotations. To do so, click on  **Set Default Settings** in the **Annotations** group.

NOTE: Set Default Settings will only set the default annotation settings for the current SlideBook session. If you restart SlideBook, the default annotations will have to be reset.



TUTORIAL: Using Regions to Graph data

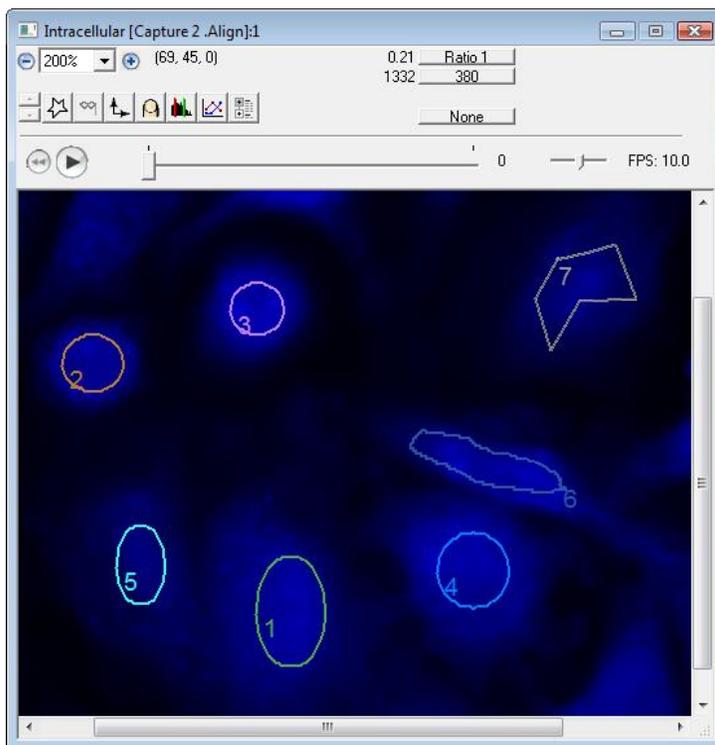
In any open image view, you may draw regions to graph channel intensity information. A drawn region will exist in all z-planes and time points of an image. This tutorial reviews drawing and editing regions, setting a background region, and graphing the region data.

1. Open a **Main View** of an image.
2. Select any of the region drawing tools from the **Region** group in the Home tab or from the tool menu in the view window.

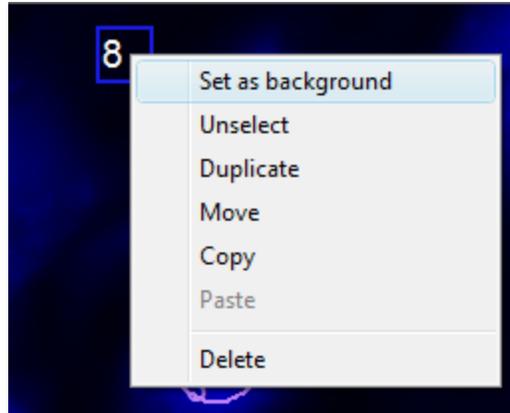


NOTE: If regions were drawn and saved with the image in the full SlideBook software, they will be displayed when you select a region drawing tool.

3. Draw regions as desired on the image by clicking and dragging. You may use any combination of the region drawing tools. When using the  **Polygon** tool, double click to close the shape.



4. If desired, draw a region in the background area of the image. Right-click and select **Set as Background** from the menu.

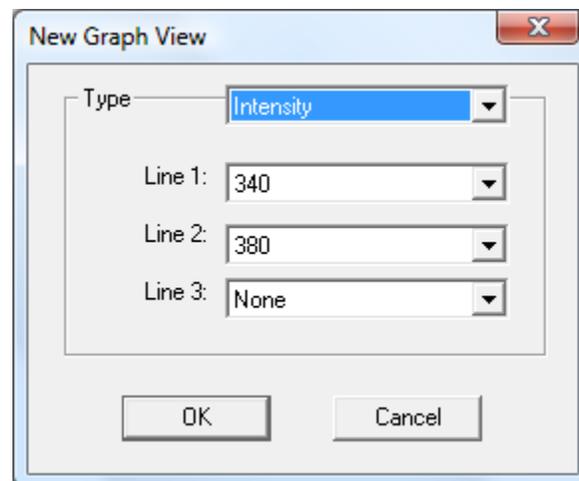


5. To select a region, click within its borders. Once a region is selected, you may drag to to change the location, press **Delete** to remove it, or right-click to copy/paste it.

6. Once you are satisfied with the regions, you may graph the data by going to  **Graph** in the **Analyze** tab or by selecting the **Graph** icon  in the view window.

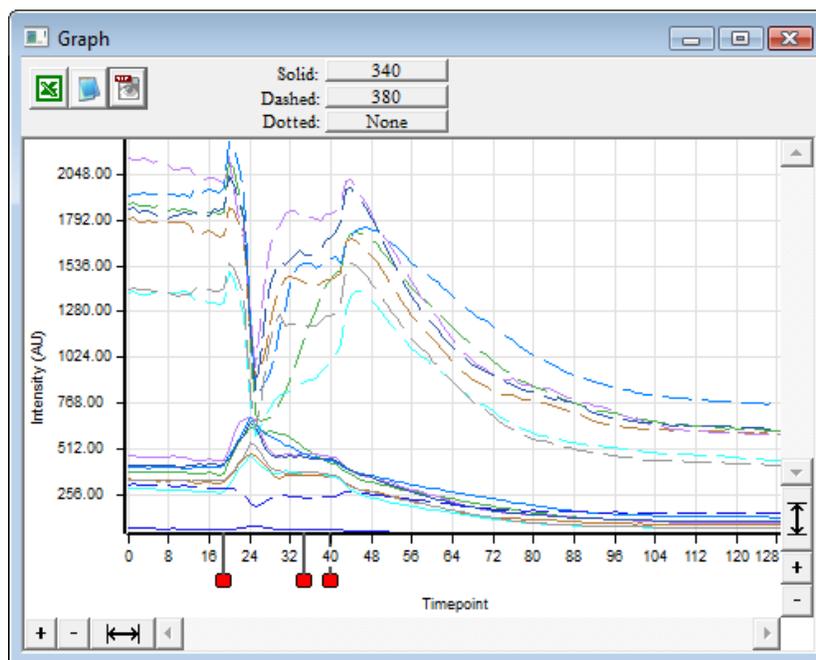
NOTE: Graph is only available when there are intensity changes over time in 2D Timelapse and 4D data types (not available for 3D or 2D images). For images that do not have a time aspect, you may use **Masks** for analysis.

7. Select the type of intensity and which channels you would like to graph from the **New Graph View** window. Click **OK**.





8. View and explore the **Graph** window.



9. If desired, export an  Excel file,  text file, or  tiff of the graph data.